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Dated 7 January 2000

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

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1.	Your reference	PHM 98-150
2.	Patent application number *(The Patent Office will fill in this part)*	**9902461.4** -5 FEB 1999
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	Zeneca Limited 15 Stanhope Gate LONDON W1Y 6LN, GB
	Patents ADP number *(if you know it)*	6254007002
	If the applicant is a corporate body, give the country/state of its incorporation	
4.	Title of the invention	CHEMICAL COMPOUNDS
5.	Name of your agent *(if you have one)*	DENERLEY, Paul Millington
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	ZENECA Pharmaceuticals Intellectual Property Department Mereside, Alderley Park, Macclesfield, Cheshire, Sk10 4TG, GB
	Patents ADP number *(if you know it)*	1030618002

		Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number			

		Number of earlier application	Date of filing *(day / month / year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application		

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	28
Claim(s)	2
Abstract	
Drawing(s)	

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	

11. I/We request the grant of a patent on the basis of this application.

Signature Lynda May Slack Date February 1999

12. Name and daytime telephone number of person to contact in the United Kingdom — Mrs Lynda May Slack 01625 516173

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Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

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c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

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CHEMICAL COMPOUNDS

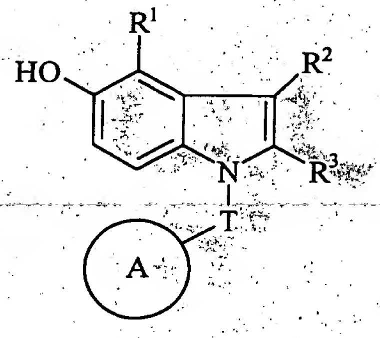
The present invention relates to anti-inflammatory compounds that act via antagonism of the CCR2 receptor, (also known as the MCP-1 receptor), leading *inter alia* to inhibition of Monocyte Chemoattractant Protein-1 (MCP-1). These compounds contain an indole moiety. The invention further relates to pharmaceutical compositions containing them, processes for their preparation, intermediates useful in their preparation and to their use as therapeutic agents.

MCP-1 is a member of the chemokine family of pro-inflammatory proteins which mediate leukocyte chemotaxis and activation. MCP-1 is a C-C chemokine which is one of the most potent and selective T-cell and monocyte chemoattractant and activating agents known. MCP-1 has been implicated in the pathophysiology of a large number of inflammatory diseases including rheumatoid arthritis, glomerular nephritides, lung fibrosis, restenosis (International Patent Application WO 94/09128), alveolitis (Jones et al., 1992, *J. Immunol.*, **149**, 2147) and asthma. Other disease areas where MCP-1 is thought to play a part in their pathology are atherosclerosis (e.g. Koch et al., 1992, *J. Clin. Invest.*, **90**, 772 -779), psoriasis (Deleuran et al., 1996, *J. Dermatological Science*, **13**,. 228-236), delayed-type hypersensitivity reactions of the skin, inflammatory bowel disease (Grimm et al., 1996, *J. Leukocyte Biol.*, **59**,. 804-812), multiple sclerosis and brain trauma (Berman et al, 1996, *J. Immunol.*, **156**,. 3017-3023). An MCP-1 inhibitor may also be useful to treat stroke, reperfusion injury, ischemia, myocardial infarction and transplant rejection.

MCP-1 acts through the CCR2 receptor. MCP-2 and MCP-3 may also act, at least in part, through this receptor. Therefore in this specification, when reference is made to "inhibition or antagonism of MCP-1" or "MCP-1 mediated effects" this includes inhibition or antagonism of MCP-2 and/or MCP-3 mediated effects when MCP-2 and/or MCP-3 are acting through the CCR2 receptor.

The applicants have found a class of compounds containing an indole moiety which have useful inhibitory activity against MCP-1. Co-pending application UK 9716657.3 discloses a class of indoles with MCP-1 inhibitory activity. This application is based on the surprising discovery that particular substituted 5-hydroxy indoles are MCP-1 inhibitors which possess unexpected and beneficial properties with respect to potency and/or blood levels and/or bioavailability and/or solubility.

Accordingly, the present invention provides a compound of the formula (I):



(I)

wherein:

R^1 is hydrogen, halo or methoxy;

R^2 is hydrogen, halo, methyl, ethyl or methoxy;

R^3 is carboxy, tetrazolyl or $-CONHSO_2R^4$ where R^4 is methyl, ethyl, phenyl, 2,5-dimethylisoxazolyl or trifluoromethyl;

T is $-CH_2-$ or $-SO_2-$; and

Ring A is 3-chlorophenyl, 4-chlorophenyl, 3-trifluoromethylphenyl, 3,4-dichlorophenyl, 3,4-difluorophenyl, 3-fluoro-4-chlorophenyl, 3-chloro-4-fluorophenyl or 2,3-dichloropyrid-5-yl;

or a pharmaceutically acceptable salt or prodrug thereof.

In this specification the term "alkyl" includes both straight and branched chain alkyl groups but references to individual alkyl groups such as "propyl" are specific for the straight chain version only. The term "halo" refers to fluoro, chloro, bromo and iodo.

Particular novel compounds of the invention include, for example, compounds of the formula (I), or pharmaceutically-acceptable salts or prodrugs thereof, wherein, unless otherwise stated:

a) R^1 has any of the values defined in i) - iii) hereinafter or a combination of two or more of these values;

b) R^2 has any of the values defined in iv) - viii) hereinafter or a combination of two or more of these values;

c) R^3 has any of the values defined in ix) - xi) hereinafter or a combination of two or more of these values;

e) T has any of the values defined in xii) - xiii) hereinafter or both of these values;

f) Ring A has any of the values defined in xiv) - xxi) hereinafter or a combination of two or more of these values;

i) R^1 is hydrogen;

ii) R^1 is halo;

iii) R^1 is methoxy;

iv) R^2 is hydrogen;

v) R^2 is halo;

vi) R^2 is methyl;

vii) R^2 is ethyl;

viii) R^2 is methoxy;

ix) R^3 is carboxy;

x) R^3 is tetrazolyl;

xi) R^3 is $-CONHSO_2R^4$ where R^4 is methyl, ethyl, phenyl, 2,5-dimethylisoxazolyl or trifluoromethyl;

xii) T is $-CH_2-$;

xiii) T is $-SO_2-$;

xiv) Ring A is 3-chlorophenyl;

xv) Ring A is 4-chlorophenyl;

xvi) Ring A is 3-trifluoromethylphenyl;

xvii) Ring A is 3,4-dichlorophenyl;

xviii) Ring A is 3,4-difluorophenyl;

xix) Ring A is 3-fluoro-4-chlorophenyl;

xx) Ring A is 3-chloro-4-fluorophenyl; and

xxi) Ring A is 2,3-dichloropyrid-5-yl.

Preferably R^1 is hydrogen.

Preferably R^2 is hydrogen.

Preferably R^3 is carboxy.

Preferably T is $-CH_2-$.

Preferably Ring A is 3-chlorophenyl, 4-chlorophenyl, 3-trifluoromethylphenyl, 3,4-dichlorophenyl, 3,4-difluorophenyl, 3-fluoro-4-chlorophenyl or 3-chloro-4-fluorophenyl.

More preferably Ring A is 3,4-dichlorophenyl or 3-chloro-4-fluorophenyl.

In another aspect of the invention preferably Ring A is 3,4-dichlorophenyl, 2,3-dichloropyrid-5-yl or 3-chloro-4-fluorophenyl.

Therefore, in a preferred aspect of the invention there is provided a compound of formula (I) as depicted above wherein:

R^1 is hydrogen;

R^2 is hydrogen;

R^3 is carboxy;

T is $-CH_2-$; and

Ring A is 3,4-dichlorophenyl or 3-chloro-4-fluorophenyl;

or a pharmaceutically acceptable salt or prodrug thereof.

Preferred compounds of the invention include any one of the Examples. More preferred compounds of the invention are Examples 1 and 3.

The invention further relates to all tautomeric forms of the compounds of formula (I).

It is also to be understood that certain compounds of the formula (I) can exist in solvated as well as unsolvated forms such as, for example, hydrated forms. It is to be understood that the invention encompasses all such solvated forms.

Compounds of formula (I) are inhibitors of monocyte chemoattractant protein-1. In addition, they appear to inhibit RANTES induced chemotaxis. RANTES (Regulated upon Activation, Normal T-cell Expressed and Secreted) is another chemokine from the same family as MCP-1, with a similar biological profile, but acting though the CCR1 receptor. As a result, these compounds can be used to treat disease mediated by these agents, in particular inflammatory disease.

Suitable pharmaceutically acceptable salts of compounds of formula (I) include base salts such as an alkali metal salt for example sodium, an alkaline earth metal salt for example calcium or magnesium, an organic amine salt for example triethylamine, morpholine, *N*-methylpiperidine, *N*-ethylpiperidine, procaine, dibenzylamine, *N*,*N*-dibenzylethylamine or amino acids for example lysine. In another aspect, where the compound is sufficiently basic, suitable salts include acid addition salts such as methanesulphonate, fumarate, hydrochloride, hydrobromide, citrate, maleate and salts formed with phosphoric and sulphuric acid. There may be more than one cation or anion depending on the number of charged functions and the valency of the cations or anions. A preferred pharmaceutically acceptable salt is a sodium salt.

Various forms of prodrugs are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology, Vol. 42, p. 309-396, edited by K. Widder, *et al.* (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen and H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p. 113-191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8, 1-38 (1992);

d) H. Bundgaard, *et al.*, Journal of Pharmaceutical Sciences, 77, 285 (1988); and

e) N. Kakeya, *et al.*, Chem Pharm Bull, 32, 692 (1984).

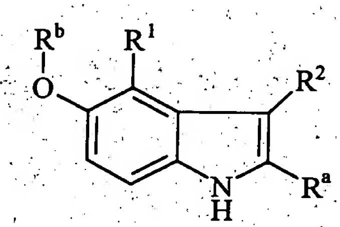
Examples of such prodrugs are *in vivo* cleavable esters of a compound of the invention. An *in vivo* cleavable ester of a compound of the invention containing a carboxy group is, for example, a pharmaceutically-acceptable ester which is cleaved in the human or animal body to produce the parent acid. Suitable pharmaceutically-acceptable esters for carboxy include C_{1-6}alkyl esters, for example methyl or ethyl; C_{1-6}alkoxymethyl esters, for example methoxymethyl; C_{1-6}alkanoyloxymethyl esters, for example pivaloyloxymethyl; phthalidyl esters; C_{3-8}cycloalkoxycarbonyloxyC_{1-6}alkyl esters, for example 1-cyclohexylcarbonyloxyethyl; 1,3-dioxolan-2-ylmethyl esters, for example 5-methyl-1,3-dioxolan-2-ylmethyl; C_{1-6}alkoxycarbonyloxyethyl esters, for example 1-methoxycarbonyloxyethyl; aminocarbonylmethyl esters and mono- or di- N-(C_{1-6}alkyl) versions thereof, for example N,N-dimethylaminocarbonylmethyl esters and N-ethylaminocarbonylmethyl esters; and may be formed at any carboxy group in the compounds of this invention. An *in vivo* cleavable ester of a compound of the invention containing a hydroxy group is, for example, a pharmaceutically-acceptable ester which is cleaved in the human or animal body to produce the parent hydroxy group. Suitable pharmaceutically acceptable esters for hydroxy include C_{1-6}alkanoyl esters, for example acetyl esters; and benzoyl esters wherein the phenyl group may be substituted with aminomethyl or N- substituted mono- or di- C_{1-6}alkyl aminomethyl, for example 4-aminomethylbenzoyl esters and 4-N,N-dimethylaminomethylbenzoyl esters.

Further examples of such prodrugs are *in vivo* cleavable amides of a compound of the invention. Examples of such *in vivo* cleavable amides include an N-C_{1-6}alkylamide and an

N,N-di-(C_{1-6}alkyl)amide such as N-methyl, N-ethyl, N-propyl, N,N-dimethyl, N-ethyl-N-methyl or N,N-diethylamide.

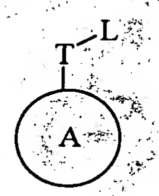
Another aspect of the present invention provides a process for preparing a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof which process (wherein R^1, R^2, R^3, T and Ring A are as defined for formula (I) unless otherwise stated) comprises of:

a) reacting compounds of formula (II):



(II)

where R^a is R^3 or protected R^3, and R^b is hydrogen or a suitable hydroxy protecting group, with a compound of formula (III):



(III)

where L is a displaceable group;

and thereafter if necessary:

i) converting a compound of the formula (I) into another compound of the formula (I);

ii) removing any protecting groups; or

iii) forming a pharmaceutically acceptable salt or prodrug thereof.

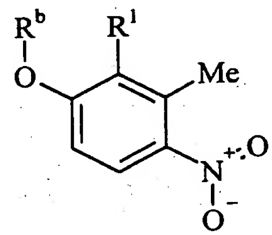
Suitable values for L are for example, a halogeno or sulphonyloxy group, for example a chloro, bromo, methanesulphonyloxy or toluene-4-sulphonyloxy group.

Specific reaction conditions for the above reactions are as follows.

a) Compounds of formula (II) and (III) may be reacted together in an inert solvent and a base such as *N,N*-dimethylformamide/sodium hydride or dichloromethane/sodium hydroxide or acetonitrile/potassium carbonate, in the presence of a phase transfer catalyst such as tetra-*n*-butylammonium hydrogensulphate for 1-6 hours preferably 1-3 hours, at a temperature of 15-30°C, preferably 20-25°C to give a compound of formula (I).

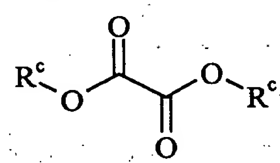
Compounds of formula **(II)** may be commercially available, or they may be made by modification using known processes of commercially available compounds of formula **(II)**, or they may be prepared by the following processes:

i) Reacting a compound of formula **(IV)**:



(IV)

where R^b is as defined above with a compound of formula **(V)**

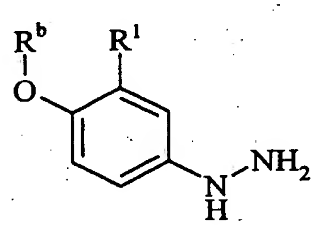


(V)

where R^c is C_{1-4}alkyl.

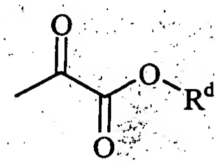
Compounds of formula **(IV)** and **(V)** are reacted together under Reissert reaction conditions such as in an inert solvent (such as tetrahydrofuran), in the presence of a base (such as potassium ethoxide), at a temperature range of 15-30°C preferably 20-25°C, for 10-20 hours preferably 15-17 hours. The resulting compound is isolated and dissolved in an alcohol such as ethanol and an organic acid (such as acetic acid) and a transition metal catalyst (such as 10% Pd/C) and cyclohexene is added. The mixture is heated at a temperature of 60-120°C preferably at 70-90°C for 15-25 hours preferably 16-20 hours to give a compound of formula **(II)** wherein R^a is $-CO_2C_{1-4}$alkyl; or

ii) Reacting a compound of formula **(VI)**:



(VI)

where R^b is as defined above, with a compound of formula **(VII)**:



(VII)

where R^d is C_{1-4}alkyl.

Compounds of formula **(VI)** and **(VII)** are reacted together under Fischer conditions such as with an organic acid (such as acetic acid), in an alcohol (such as ethanol), at a temperature of 60-90°C, preferably 75-85°C, for 1-5 hours, preferably 1-3 hours. The resulting compound is mixed with a strong acid (such as polyphosphoric acid) and heated at 90-150°C preferably 100-120°C, for 0.5-4 hours, preferably 0.5-2 hours to give a compound of formula **(II)** in which R^2 is hydrogen. Then, if desired, R^2 can be optionally converted into another value of R^2 as defined in formula **(I)** using techniques known in the art such as those described below.

Compounds of formula **(III)**, **(IV)**, **(V)**, **(IV)** and **(VII)** are known or commercially available or are prepared by processes known in the art by standard manipulation of commercially available or known materials.

R^c and R^d are C_{1-4}alkyl. Preferably R^c and R^d are methyl or ethyl.

It will also be appreciated that in some of the reactions mentioned herein it may be necessary/desirable to protect any sensitive groups in the compounds. The instances where protection is necessary or desirable and suitable methods for protection are known to those skilled in the art. Thus, if reactants include groups such as amino, carboxy or hydroxy it may be desirable to protect the group in some of the reactions mentioned herein.

A suitable protecting group for an amino or alkylamino group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an alkoxycarbonyl group, for example a methoxycarbonyl, ethoxycarbonyl or *t*-butoxycarbonyl group, an arylmethoxycarbonyl group, for example benzyloxycarbonyl, or an aroyl group, for example benzoyl. The deprotection conditions for the above protecting groups necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or alkoxycarbonyl group or an aroyl group may be removed for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an acyl group such as a *t*-butoxycarbonyl group may be removed, for example, by treatment with a suitable acid

as hydrochloric, sulphuric or phosphoric acid or trifluoroacetic acid and an arylmethoxycarbonyl group such as a benzyloxycarbonyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon, or by treatment with a Lewis acid for example boron tris(trifluoroacetate). A suitable alternative protecting group for a primary amino group is, for example, a phthaloyl group which may be removed by treatment with an alkylamine, for example dimethylaminopropylamine, or with hydrazine.

A suitable protecting group for a hydroxy group is, for example, an acyl group, for example an alkanoyl group such as acetyl, an aroyl group, for example benzoyl, or an arylmethyl group, for example benzyl. The deprotection conditions for the above protecting groups will necessarily vary with the choice of protecting group. Thus, for example, an acyl group such as an alkanoyl or an aroyl group may be removed, for example, by hydrolysis with a suitable base such as an alkali metal hydroxide, for example lithium or sodium hydroxide. Alternatively an arylmethyl group such as a benzyl group may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

A suitable protecting group for a carboxy group is, for example, an esterifying group, for example a methyl or an ethyl group which may be removed, for example, by hydrolysis with a base such as sodium hydroxide, or for example a *t*-butyl group which may be removed, for example, by treatment with an acid, for example an organic acid such as trifluoroacetic acid, or for example a benzyl group which may be removed, for example, by hydrogenation over a catalyst such as palladium-on-carbon.

The protecting groups may be removed at any convenient stage in the synthesis using conventional techniques well known in the chemical art.

Some of the intermediates described herein may be novel, for example intermediates of the formula **(II)**, and as such they are provided as a further feature of the invention.

When a pharmaceutically-acceptable salt of a compound of formula **(I)** is required, it may be obtained, for example, by reaction of said compound with the appropriate acid (which affords a physiologically acceptable anion), or with the appropriate base (which affords a physiologically acceptable cation), or by any other conventional salt formation procedure.

According to a further aspect of the invention there is provided a pharmaceutical composition which comprises a compound of the formula **(I)** as defined hereinbefore or a pharmaceutically acceptable salt or prodrug thereof, in association with a pharmaceutically acceptable excipient or carrier.

The compositions of the invention may be in a form suitable for oral use (for example as tablets, lozenges, hard or soft capsules, aqueous or oily suspensions, emulsions, dispersible powders or granules, syrups or elixirs), for topical use (for example as creams, ointments, gels, or aqueous or oily solutions or suspensions), for administration by inhalation (for example as a finely divided powder or a liquid aerosol), for administration by insufflation (for example as a finely divided powder) or for parenteral administration (for example as a sterile aqueous or oily solution for intravenous, subcutaneous, intramuscular or intramuscular dosing or as a suppository for rectal dosing).

The compositions of the invention may be obtained by conventional procedures using conventional pharmaceutical excipients, well known in the art. Thus, compositions intended for oral use may contain, for example, one or more colouring, sweetening, flavouring and/or preservative agents.

Suitable pharmaceutically acceptable excipients for a tablet formulation include, for example, inert diluents such as lactose, sodium carbonate, calcium phosphate or calcium carbonate, granulating and disintegrating agents such as corn starch or algenic acid; binding agents such as starch; lubricating agents such as magnesium stearate, stearic acid or talc; preservative agents such as ethyl or propyl p-hydroxybenzoate, and anti-oxidants, such as ascorbic acid. Tablet formulations may be uncoated or coated either to modify their disintegration and the subsequent absorption of the active ingredient within the gastrointestinal track, or to improve their stability and/or appearance, in either case, using conventional coating agents and procedures well known in the art.

Compositions for oral use may be in the form of hard gelatin capsules in which the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules in which the active ingredient is mixed with water or an oil such as peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions generally contain the active ingredient in finely powdered form together with one or more suspending agents, such as sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents such as lecithin or condensation products of an alkylene oxide with fatty acids (for example polyoxyethylene stearate), or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters

derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives (such as ethyl or propyl p-hydroxybenzoate, anti-oxidants (such as ascorbic acid), colouring agents, flavouring agents, and/or sweetening agents (such as sucrose, saccharine or aspartame).

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil (such as arachis oil, olive oil, sesame oil or coconut oil) or in a mineral oil (such as liquid paraffin). The oily suspensions may also contain a thickening agent such as beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set out above, and flavouring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water generally contain the active ingredient together with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients such as sweetening, flavouring and colouring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or arachis oil, or a mineral oil, such as for example liquid paraffin or a mixture of any of these. Suitable emulsifying agents may be, for example, naturally-occurring gums such as gum acacia or gum tragacanth, naturally-occurring phosphatides such as soya bean, lecithin, an esters or partial esters derived from fatty acids and hexitol anhydrides (for example sorbitan monooleate) and condensation products of the said partial esters with ethylene oxide such as polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening, flavouring and preservative agents.

Syrups and elixirs may be formulated with sweetening agents such as glycerol, propylene glycol, sorbitol, aspartame or sucrose, and may also contain a demulcent, preservative, flavouring and/or colouring agent.

The pharmaceutical compositions may also be in the form of a sterile injectable aqueous or oily suspension, which may be formulated according to known procedures using one or more of the appropriate dispersing or wetting agents and suspending agents, which have been mentioned above. A sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example a solution in 1,3-butanediol.

Suppository formulations may be prepared by mixing the active ingredient with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Suitable excipients include, for example, cocoa butter and polyethylene glycols.

Topical formulations, such as creams, ointments, gels and aqueous or oily solutions or suspensions, may generally be obtained by formulating an active ingredient with a conventional, topically acceptable, vehicle or diluent using conventional procedure well known in the art.

Compositions for administration by insufflation may be in the form of a finely divided powder containing particles of average diameter of, for example, 30μ or much less, the powder itself comprising either active ingredient alone or diluted with one or more physiologically acceptable carriers such as lactose. The powder for insufflation is then conveniently retained in a capsule containing, for example, 1 to 50mg of active ingredient for use with a turbo-inhaler device, such as is used for insufflation of the known agent sodium cromoglycate.

Compositions for administration by inhalation may be in the form of a conventional pressurised aerosol arranged to dispense the active ingredient either as an aerosol containing finely divided solid or liquid droplets. Conventional aerosol propellants such as volatile fluorinated hydrocarbons or hydrocarbons may be used and the aerosol device is conveniently arranged to dispense a metered quantity of active ingredient.

For further information on Formulation the reader is referred to Chapter 25.2 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The amount of active ingredient that is combined with one or more excipients to produce a single dosage form will necessarily vary depending upon the host treated and the particular route of administration. For example, a formulation intended for oral administration

to humans will generally contain, for example, from 0.5 mg to 2 g of active agent compounded with an appropriate and convenient amount of excipients which may vary from about 5 to about 98 percent by weight of the total composition. Dosage unit forms will generally contain about 1 mg to about 500 mg of an active ingredient. For further information on Routes of Administration and Dosage Regimes the reader is referred to Chapter 25.3 in Volume 5 of Comprehensive Medicinal Chemistry (Corwin Hansch; Chairman of Editorial Board), Pergamon Press 1990.

The size of the dose for therapeutic or prophylactic purposes of a compound of the Formula I will naturally vary according to the nature and severity of the conditions, the age and sex of the animal or patient and the route of administration, according to well known principles of medicine. As mentioned above, compounds of the Formula I are useful in treating diseases or medical conditions which are due alone or in part to the effects of farnesylation of rats.

In using a compound of the Formula I for therapeutic or prophylactic purposes it will generally be administered so that a daily dose in the range, for example, 0.5 mg to 75 mg per kg body weight is received, given if required in divided doses. In general lower doses will be administered when a parenteral route is employed. Thus, for example, for intravenous administration, a dose in the range, for example, 0.5 mg to 30 mg per kg body weight will generally be used. Similarly, for administration by inhalation, a dose in the range, for example, 0.5 mg to 25 mg per kg body weight will be used. Oral administration is however preferred.

According to a further aspect of the present invention there is provided a compound of the formula (I) or a pharmaceutically acceptable salt or prodrug thereof, as defined hereinbefore for use in a method of treatment of the human or animal body by therapy. Conveniently, the invention provides a method of treating inflammatory disease by administering a compound of formula (I) or a pharmaceutically acceptable salt or prodrug or a pharmaceutical composition thereof, as described above.

A further feature of the present invention is a compound of formula (I) and pharmaceutically acceptable salt or prodrug thereof, for use as a medicament.

Conveniently this is a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof, for use as a medicament for antagonising an MCP-1 mediated effect in a warm-blooded animal such as a human being.

Thus according to a further aspect of the invention there is provided the use of a compound of the formula (I), or a pharmaceutically acceptable salt or prodrug thereof, in the manufacture of a medicament for use in antagonising an MCP-1 mediated effect in a warm-blooded animal such as a human being.

According to a further feature of the invention there is provided a method of antagonising an MCP-1 mediated effect in a warm-blooded animal, such as a human being, in need of such treatment which comprises administering to said animal an effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof, as defined hereinbefore.

Biological Testing.

The following biological test methods, data and Examples serve to illustrate the present invention.

Abbreviations:

ATCC	American Type Culture Collection, Rockville, USA.
BCA	Bicinchroninic acid, (used, with copper sulphate, to assay protein)
BSA	Bovine Serum Albumin
DMEM	Dulbecco's modified Eagle's medium
EGTA	Ethylenebis(oxyethylenenitrilo)tetraacetic acid
FCS	Foetal calf serum
HEPES	(N-[2-Hydroxyethyl]piperazine-N'-[2-ethanesulphonic acid])
HBSS	Hank's Balanced Salt Solution
hMCP-1	Human Monocyte Chemoattractant Protein-1
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction

AMPLITAQ™, available from Perkin-Elmer Cetus, is used as the source of thermostable DNA polymerase.

Binding Buffer is 50 mM HEPES, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% foetal calf serum, adjusted to pH 7.2 with 1 M NaOH.

Non-Essential Amino Acids (100X concentrate) is: L-Alanine, 890 mg/l; L-Asparagine, 1320 mg/l; L-Aspartic acid, 1330 mg/l; L-Glutamic acid, 1470 mg/l; Glycine, 750 mg/l; L-Proline, 1150 mg/l and; L-Serine, 1050 mg/l.

Hypoxanthine and Thymidine Supplement (50x concentrate) is: hypoxanthine, 680 mg/l and; thymidine, 194 mg/l.

Penicillin-Streptomycin is: Penicillin G (sodium salt); 5000 units/ml; Streptomycin sulphate, 5000 μg/ml.

Human monocytic cell line THP-1 cells are available from ATCC, accession number ATCC TIB-202.

Hank's Balanced Salt Solution (HBSS) was obtained from Gibco; see *Proc. Soc. Exp. Biol. Med.*, 1949, **71**, 196.

Synthetic cell culture medium, RPMI 1640 was obtained from Gibco; it contains inorganic salts [$Ca(NO_3)_2.4H_2O$ 100 mg/l; KCl 400 mg/l; $MgSO_4.7H_2O$ 100 mg/l; NaCl 6000 mg/l; $NaHCO_3$ 2000 mg/l & Na_2HPO_4 (anhyd) 800 mg/l], D-Glucose 2000 mg/l, reduced glutathione 1 mg/l, amino acids and vitamins.

FURA-2/AM is 1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxy]-2-(2'-amino-5'-methylphenoxy)-ethane-*N,N,N',N'*-tetraacetic acid pentaacetoxymethyl ester and was obtained from Molecular Probes, Eugene, Oregon, USA.

Blood Sedimentation Buffer contains 8.5g/l NaCl and 10g/l hydroxyethyl cellulose.

Lysis Buffer is 0.15M NH_4Cl^-, 10mM $KHCO_3$, 1mM EDTA

Whole Cell Binding Buffer is 50 mM HEPES, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.5% BSA, 0.01% NaN_3, adjusted to pH 7.2 with 1M NaOH.

Wash buffer is 50mM HEPES. 1mM $CaCl_2$, 5mM $MgCl_2$, 0.5% heat inactivated FCS, 0.5MNaCl adjusted to pH7.2 with 1M NaOH.

General molecular biology procedures can be followed from any of the methods described in "Molecular Cloning - A Laboratory Manual" Second Edition, Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory, 1989).

i) Cloning and expression of hMCP-1 receptor

The MCP-1 receptor B (CCR2B) cDNA was cloned by PCR from THP-1 cell RNA using suitable oligonucleotide primers based on the published MCP-1 receptor sequences (Charo *et al.*, 1994, *Proc. Natl. Acad. Sci. USA*, **91**, 2752). The resulting PCR products were cloned into vector PCR-II™ (InVitrogen, San Diego, CA.). Error free CCR2B cDNA was subcloned as a Hind III-Not I fragment into the eukaryotic expression vector pCDNA3 (InVitrogen) to generate pCDNA3/CC-CKR2A and pCDNA3/CCR2B respectively.

Linearised pCDNA3/CCR2B DNA was transfected into CHO-K1 cells by calcium phosphate precipitation (Wigler *et al.*, 1979, *Cell*, **16**, 777). Transfected cells were selected by the addition of Geneticin Sulphate (G418, Gibco BRL) at 1mg/ml, 24 hours after the cells had been transfected. Preparation of RNA and Northern blotting were carried out as described previously (Needham *et al.*, 1995, *Prot. Express. Purific.*, **6**, 134). CHO-K1 clone 7 (CHO-CCR2B) was identified as the highest MCP-1 receptor B expressor.

<u>ii) Preparation of membrane fragments</u>

CHO-CCR2B cells were grown in DMEM supplemented with 10% foetal calf serum, 2 mM glutamine, 1x Non-Essential Amino Acids, 1x Hypoxanthine and Thymidine Supplement and Penicillin-Streptomycin (at 50 μg streptomycin/ml, Gibco BRL). Membrane fragments were prepared using cell lysis/differential centrifugation methods as described previously (Siciliano *et al.*, 1990, *J. Biol. Chem.*, **265**, 19658). Protein concentration was estimated by BCA protein assay (Pierce, Rockford, Illinois) according to the manufacturer's instructions.

<u>iii) Assay</u>

^{125}I MCP-1 was prepared using Bolton and Hunter conjugation (Bolton *et al.*, 1973, *Biochem. J.*, **133**, 529; Amersham International plc]. Equilibrium binding assays were carried out using the method of Ernst *et al.*, 1994, *J. Immunol.*, **152**, 3541. Briefly, varying amounts of ^{125}I-labeled MCP-1 were added to 7μg of purified CHO-CCR2B cell membranes in 100 μl of Binding Buffer. After 1 hour incubation at room temperature the binding reaction mixtures were filtered and washed 5 times through a plate washer (Brandel MLR-96T Cell Harvester) using ice cold Binding Buffer. Filter mats (Brandel GF/B) were pre-soaked for 60 minutes in 0.3% polyethylenimine prior to use. Following filtration individual filters were separated into 3.5ml tubes (Sarstedt No. 55.484) and bound ^{125}I-labeled MCP-1 was determined (LKB 1277 Gammamaster). Cold competition studies were performed as above using 100 pM ^{125}I-labeled MCP-1 in the presence of varying concentrations of unlabelled MCP-1. Non-specific binding was determined by the inclusion of a 200-fold molar excess of unlabelled MCP-1 in the reaction.

Ligand binding studies with membrane fragments prepared from CHO-CCR2B cells showed that the CCR2B receptor was present at a concentration of 0.2 pmoles/mg of membrane protein and bound MCP-1 selectively and with high affinity (IC_{50} = 110 pM, K_d =120 pM). Binding to these membranes was completely reversible and reached equilibrium

after 45 minutes at room temperature, and there was a linear relationship between MCP-1 binding and CHO-CCR2B cell membrane concentration when using MCP-1 at concentrations between 100 pM and 500 pM.

Test compounds dissolved in DMSO (5µl) were tested in competition with 100 pM labelled MCP-1 over a concentration range (0.01-50µM) in duplicate using eight point dose-response curves and IC_{50} concentrations were calculated.

Compounds tested of the present invention had IC_{50} values of 50µM or less in the hMCP-1 receptor binding assay described herein.

b) MCP-1 mediated calcium flux in THP-1 cells

The human monocytic cell line THP-1 was grown in a synthetic cell culture medium RPMI 1640 supplemented with 10 % foetal calf serum, 6mM glutamine and Penicillin-Streptomycin (at 50 µg streptomycin/ml, Gibco BRL). THP-1 cells were washed in HBSS (lacking Ca^{2+} and Mg^{2+}) + 1 mg/ml BSA and resuspended in the same buffer at a density of 3 x 10^6 cells/ml. The cells were then loaded with 1mM FURA-2/AM for 30 min at 37°C, washed twice in HBSS, and resuspended at 1x10^6 cells/ml. THP-1 cell suspension (0.9 ml) was added to a 5 ml disposable cuvette containing a magnetic stirrer bar and 2.1 ml of prewarmed (37°C) HBSS containing 1 mg/ml BSA, 1 mM $MgCl_2$ and 2 mM $CaCl_2$. The cuvette was placed in a fluorescence spectrophotometer (Perkin Elmer, Norwalk, CT) and preincubated for 4 min at 37°C with stirring. Fluorescence was recorded over 70 sec and cells were stimulated by addition of hMCP-1 to the cuvette after 10 sec. $[Ca^{2+}]i$ was measured by excitation at 340 nm and 380 nm alternately and subsequent measurement of the intensity of the fluorescence emission at 510 nm. The ratio of the intensities of the emitted fluorescent light following excitation at 340 nm and 380 nm, (R), was calculated and displayed to give and estimate of cytoplasmic $[Ca^{2+}]$ according to the equation:-

$$[Ca^{2+}]i = K_d \frac{(R-Rmin)}{(Rmax-R)} (Sf2/Sb2)$$

where the K_d for FURA-2 Ca^{2+} complex at 37°C was taken to be 224nm. R_{max} is the maximal fluorescence ratio determined after addition of 10 mM Ionomycin, R_{min} is the minimal ratio determined by the subsequent addition of a Ca^{2+} free solution containing 5 mM EGTA, and Sf2/Sb2 is the ratio of fluorescence values at 380 nm excitation determined at R_{min} and R_{max}, respectively.

Stimulation of THP-1 cells with hMCP-1 induced a rapid, transient rise in $[Ca^{2+}]_i$ in a specific and dose dependent manner. Dose response curves indicated an approximate EC_{50} of 2 nm. Test compounds dissolved in DMSO (10μl) were assayed for inhibition of calcium release by adding them to the cell suspension 10 sec prior to ligand addition and measuring the reduction in the transient rise in $[Ca^{2+}]i$. Test compounds were also checked for lack of agonist activity by addition in place of hMCP-1.

c) hMCP-1 and RANTES mediated chemotaxis.

In vitro chemotaxis assays were performed using the human monocytic cell line THP-1. Cell migration through polycarbonate membranes was measured by enumerating those passing through either directly by Coulter counting or indirectly by use of a colourimetric viability assay measuring the cleavage of a tetrazolium salt by the mitochondrial respiratory chain (Scudiero D.A. *et al.* 1988, *Cancer Res.*, **48**, 4827-4833).

Chemoattractants were introduced into a 96-well microtitre plate which forms the lower well of a chemotaxis chamber fitted with a PVP-free 5 μm poresize polycarbonate adhesive framed filter membrane (NeuroProbe MB series, Cabin John, MD 20818, USA) according to the manufacturer's instructions. The chemoattractant was diluted as appropriate in synthetic cell culture medium, RPMI 1640 (Gibco) or supplemented with 2 mM glutamine and 0.5% BSA, or alternatively with HBSS with Ca^{2+} and Mg^{2+} without Phenol Red (Gibco) plus 0.1% BSA. Each dilution was degassed under vacuum for 30 min and was placed (400 μl) in the lower wells of the chamber and THP-1 cells ($5x10^5$ in 100 μl RPMI 1640 + 0.5%BSA) were incubated in each well of the upper chamber. For the inhibition of chemotaxis the chemoattractant was kept at a constant submaximal concentration determined previously (1nM MCP-1) and added to the lower well together with the test compounds dissolved in DMSO (final DMSO concentration < 0.05% v/v) at varying concentrations. The chamber was incubated for 2 h at 37°C under 5 % CO_2. The medium was removed from the upper wells which were then washed out with 200 μl physiological saline before opening the chamber, wiping dry the membrane surface and centrifuging the 96-well plate at 600 g for 5 min to harvest the cells. Supernatant (150 μl) was aspirated and 10 μl of cell proliferation reagent, WST-1, {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-phenyl disulfonate} plus an electron coupling reagent (Boehringer Mannheim, Cat.no. 1644 807) was added back to the wells. The plate was incubated at 37°C for 3 h and the absorbance of the soluble formazan product was read on a microtitre plate reader at 450 nm. The data was input

into a spreadsheet, corrected for any random migration in the absence of chemoattractant and the average absorbance values, standard error of the mean, and significance tests were calculated. hMCP-1 induced concentration dependent cell migration with a characteristic biphasic response, maximal 0.5-1.0 nm.

In an alternative form of the above assay, fluorescently tagged cells can be used in order to assist in end point detection. In this case, the THP-1 cells used are fluorescently tagged by incubation in the presence of 5mM Calcein AM (Glycine, N,N'-[[3',6'-bis(acetyloxy)-3-oxospiro[isobenzofuran-1(3H),9'-[9H]xanthene]-2',7'-diyl]bis(methylene)] bis[N-[2-[(acetyloxy)methoxy]-2-oxoethyl]]-bis[(acetyloxy)methyl] ester; Molecular Probes) for 45 minutes in the dark. Cells are harvested by centrifugation and resuspended in HBSS (without Phenol Red) with Ca^{2+}, Mg^{2+} and 0.1% BSA. 50μl (2x105 cells) of the cell suspension are placed on the filter above each well and, as above, the unit is incubated at 37°C for 2 hours under 5% CO_2. At the end of the incubation, cells are washed off the upper face of the filter with phosphate buffered saline, the filter removed from the plate and the number of cells attracted to either the underside of the filter or the lower well estimated by reading fluorescence at 485nm excitation, 538nm emission wavelengths (fmax, Molecular Devices). The data was input into a spreadsheet, corrected for any random migration in the absence of chemoattractant and the average fluorescence values, standard error of the mean, percentage inhibition and IC_{50} of compounds under test and significance tests can be calculated. In addition to MCP-1 induced chemotaxis, this alternative form of the assay was also used to measure inhibition of RANTES (2nM) induced chemotaxis.

d) Binding to human peripheral blood mononuclear cells(PBMCs)

i) Preparation of human PBMCs

Fresh human blood (200ml) was obtained from volunteer donors, collected into sodium citrate anticoagulant to give a final concentration of 0.38%. The blood was mixed with Sedimentation Buffer and incubated at 37°C for 20 minutes. The supernatant was collected and centrifuged at 1700rpm for 5 minutes (Sorvall RT6000D). The pellet obtained was resuspended in 20 ml RPMI/BSA (1mg/ml) and 4 x 5mls of cells were carefully layered over 4 x 5mls of Lymphoprep™ (Nycomed) in 15ml centrifuge tubes. Tubes were spun at 1700rpm for 30 minutes (Sorvall RT6000D) and the resultant layer of cells was removed and transferred to 50ml Falcon tubes. The cells were washed twice in Lysis Buffer to remove any remaining red blood cells followed by 2 washes in RPMI/BSA. Cells were resuspended in

5mls of Binding Buffer. Cell number was measured on a Coulter counter and additional binding buffer was added to give a final concentration of $1.25x10^7$ PBMCs /ml.

ii) Assay

[^{125}I]MCP-1 was prepared using Bolton and Hunter conjugation (Bolton *et al.*, 1973, *Biochem. J.*, **133**, 529; Amersham International plc]. Equilibrium binding assays were carried out using the method of Ernst *et al.*, 1994, *J. Immunol.*, **152**, 3541. Briefly, 50μl of ^{125}I-labeled MCP-1 (final concentration 100pM) was added to 40μl ($5x10^5$ cells) of cell suspension in a 96 well plate. Compounds, diluted in Whole Cell Binding Buffer from a stock solution of 10mM in DMSO were added in a final volume of 5μl to maintain a constant DMSO concentration in the assay of 5%. Total binding was determined in the absence of compound. Non-specific binding was defined by the addition of 5μl cold MCP-1 to give a final assay concentration of 100nM. Assay wells were made up to a final volume of 100μl with Whole Cell Binding Buffer and the plates sealed. Following incubation at 37°C for 60 minutes the binding reaction mixtures were filtered and washed for 10 seconds using ice cold Wash Buffer using a plate washer (Brandel MLR-96T Cell Harvester). Filter mats (Brandel GF/B) were pre-soaked for 60 minutes in 0.3% polyethylenimine plus 0.2% BSA prior to use. Following filtration individual filters were separated into 3.5ml tubes (Sarstedt No. 55.484) and bound ^{125}I-labeled MCP-1 was determined (LKB 1277 Gammamaster).

Test compound potency was determined by assay in duplicate using six point dose-response curves and IC_{50} concentrations were determined.

No physiologically unacceptable toxicity was observed at the effective dose for compounds tested of the present invention.

The invention is further illustrated, but not limited by the following Examples in which the following general procedures were used unless stated otherwise.

i) N,N-Dimethylformamide (DMF) was dried over 4Å molecular sieves. Anhydrous tetrahydrofuran (THF) was obtained from Aldrich SURESEAL™ bottles. Other commercially available reagents and solvents were used without further purification unless otherwise stated. Organic solvent extracts were dried over anhydrous $MgSO_4$.

ii) ^{1}H, ^{13}C and ^{19}F NMR were recorded on Bruker WM200, WM250, WM300 or WM400 instruments using DMSO-d_6 with Me_4Si or CCl_3F as internal standard as appropriate, unless otherwise stated. Chemical shifts are quoted in δ (ppm) and peak multiplicities are designated

as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplets; q, quartet; m, multiplet; br, broad.

iii) Mass spectra were recorded on VG 12-12 quadrupole, VG 70-250 SE, VG ZAB 2-SE or a VG modified AEI/Kratos MS9 spectrometers.

iv) For TLC analysis, Merck precoated TLC plates (silica gel 60 F254, d = 0.25 mm) were used.

v) Flash chromatography was performed on silica (Merck Kieselgel: Art.9385).

Example 1

N-(3,4-Dichlorobenzyl)-5-hydroxyindole-2-carboxylic acid

Sodium hydroxide (2M, 3 ml) was added to a stirred solution of ethyl *N*-(3,4-dichlorobenzyl)-5-hydroxyindole-2-carboxylate (0.1 g) in THF (3 ml) and methanol (1.5 ml). The reaction was stirred at ambient temperature for 4 hours. The reaction was concentrated *in vacuo* and the residue was dissolved in water (5 ml). The solution was acidified by the addition of aqueous hydrochloric acid (2M, 4 ml) precipitating the product as a white solid. The product was filtered, washed with water and dried in vacuo to yield the title compound (82 mg, 89%). NMR: 5.77 (s, 2H), 6.81 (dd, 1H), 6.89 (dd, 1H), 6.95 (d, 1H), 7.13 (s, 1H), 7.26 (d, 1H), 7.34 (d, 1H), 7.52 (d, 1H), 9.01 (s, 1H), 12.85 (s, 1H); m/z 334 ($M-H^+$).

The procedure described in the above example were repeated using ethyl *N*-[(2,3-dichloropyrid-5-yl)methyl]-5-acetoxyindole-2-carboxylate (Example 2) and ethyl *N*-(3-chloro-4-fluorobenzyl)-5-acetoxyindole-2-carboxylate (Example 3) as the starting materials. Thus were obtained the compounds described below.

Example 2

N-[(2,3-Dichloropyrid-5-yl)methyl]-5-hydroxyindole-2-carboxylic acid

36% yield. NMR: 5.80 (s, 2H), 6.84 (dd, 1H), 6.96 (d, 1H), 7.14 (s, 1H), 7.23 (d, 1H), 7.73 (d, 1H), 8.06 (d, 1H); m/z 339 ($M-H^+$) 337, 335.

Example 3

N-(3-Chloro-4-fluorobenzyl)-5-hydroxyindole-2-carboxylic acid

68% yield. NMR: 5.75 (s, 2H), 6.82 (dd, 1H), 6.95 (m, 2H), 7.12 (s, 1H), 7.2 - 7.4 (m, 3H); m/z 320 $(M-H^+)$, 318.

Preparation of Starting Materials

The starting materials for the Examples above are either commercially available or are readily prepared by standard methods from known materials. For example the following reactions (Methods A-E) are illustrations but not limitations of the preparation of the starting materials used in the above reactions.

Method A

3-Chloro-4-fluorobenzyl bromide

A solution of 3-chloro-4-fluorobenzaldehyde (3 g) in THF (40 ml) was added over 2 minutes to a stirred suspension of sodium borohydride (1.07 g) in methanol (40 ml) at 0°C. The mixture was allowed to warm to room temperature at which point carbon dioxide gas was bubbled through the reaction mixture for 10 minutes. The resulting suspension was partitioned between water and diethyl ether and combined organic extracts were dried and concentrated *in vacuo*. The residue was dissolved in dichloromethane (90 ml) and triphenylphosphine (4.62 g) and tetrabromomethane (6.64 g) were added at 0°C. The mixture was allowed to warm to room temperature overnight then concentrated *in vacuo* and the residue purified by column chromatography using *iso*-hexane as eluent to yield the desired product (3.57 g, 85%). NMR: 4.7 (s, 2H), 7.4 (m, 2H), 7.7 (m, 1H).

Method B

2,3-Dichloro-5-(hydroxymethyl)pyridine

Borane-tetrahydrofuran complex (1M solution in tetrahydrofuran, 52 ml) was added to a stirred solution of 5,6-dichloronicotinic acid (2 g) in tetrahydrofuran (60 ml) over 20 minutes at 0°C. The reaction mixture was allowed to warm to room temperature over 90 minutes and then cooled to 0°C and quenched with water (100 ml). The solution was saturated with solid sodium chloride and extracted with ethyl acetate and combined organic extracts were dried and concentrated *in vacuo*. The residue was triturated with dichloromethane-50%

ethyl acetate and the solid by-product was removed by filtration. The filtrate was concentrated *in vacuo* and purified by column chromatography using isohexane : 50% ethyl acetate as eluent to yield the product as a white solid (820 mg, 45%). NMR: 4.55 (d, 2H), 5.5 (t, 1H), 8.0 (m, 1H), 8.3 (m, 1H); m/z 178.1 ($M+H^+$).

Method C

2,3-Dichloro-5-(bromomethyl)pyridine

2,3-Dichloro-5-(hydroxymethyl)pyridine (275 mg) was dissolved in dichloromethane (10 ml) and stirred in the presence of triphenylphosphine (444 mg) and tetrabromomethane (641 mg) overnight. The solution was concentrated *in vacuo* and the residue purified by column chromatography using isohexane : 2.5% ethyl acetate as eluent to yield the product as a white solid (270 mg, 73%). NMR: 4.75 (s, 2H), 8.25 (m, 1H), 8.5 (m, 1H); m/z 242 ($M+H^+$).

Method D

Ethyl 5-acetoxy-*N*-(3,4-dichlorobenzyl)indole-2-carboxylate

i) Ethyl 5-hydroxyindole-2-carboxylate

Boron tribromide (64.58 g) was added dropwise to a stirred solution of ethyl 5-methoxyindole-2-carboxylate (20 g) in dichloromethane (1000 ml) at -78°C under an atmosphere of argon. The reaction was allowed to warm to room temperature and stirred for a further 2 hours. The reaction was poured into ice / saturated aqueous sodium hydrogen carbonate solution with stirring and extracted with ethyl acetate. Combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate solution, water, aqueous saturated sodium chloride solution and dried. The solution was concentrated *in vacuo* and the residue was purified by column chromatography using 0 - 60% diethyl ether; *iso*-hexane as eluent to yield product as a white solid (9.02 g, 48%). NMR: 1.31 (t, 3H), 4.29 (q, 2H), 6.79 (dd, 1H), 6.90 (dd, 1H), 7.22 (d, 1H), 8.84 (s, 1H), 11.52 (brs, 1H); m/z 206 ($M+H^+$).

ii) Ethyl 5-acetoxyindole-2-carboxylate

A stirred solution of ethyl 5-hydroxyindole-2-carboxylate (7.79 g) and 4-dimethylaminopyridine (20 mg) in acetic anhydride (80 ml) was heated at 80°C for 4 hours. The reaction was concentrated *in vacuo* and the residue was dissolved in ethyl acetate. Combined organic extracts were washed with hydrochloric acid (2 M), saturated aqueous sodium hydrogen carbonate solution, water, aqueous saturated sodium chloride solution and

dried. The solution was concentrated *in vacuo* to yield the product as a yellow solid (9.39 g, 100 %). NMR: 1.20 (t, 3H), 2.10 (s, 3H), 4.19 (q, 2H), 6.86 (dd, 1H), 6.97 (d, 1H), 7.20 (s, 1H), 7.29 (d, 1H); m/z 248 $(M+H^+)$.

iii) Ethyl 5-acetoxy-*N*-(3,4-dichlorobenzyl)indole-2-carboxylate

3,4-Dichlorobenzyl bromide (5.96 g) was added to a stirred solution of ethyl 5-acetoxyindole-2-carboxylate (5.4 g) and potassium carbonate (6.94 g) in acetonitrile (500 ml) under an atmosphere of argon. The reaction was heated at 80°C for 16 hours, then concentrated *in vacuo* and the residue partitioned between ethyl acetate and water. Combined organic extracts were washed with water, saturated aqueous sodium chloride and dried. The solvent was removed *in vacuo* and the residue was triturated with *iso*-hexane to yield the product as a cream solid (5.55 g, 63%). NMR: 1.27 (t, 3H), 2.27 (s, 3H), 4.28 (q, 2H), 5.82 (s, 2H), 6.90 (d, 1H), 7.09 (dd, 1H), 7.33 - 7.40 (m, 2H), 7.46 (d, 1H) 7.52 (d, 1H), 7.60 (d, 1H).

The procedures described in Method D i) - iii) were repeated using the appropriate benzyl halide. Thus were obtained the compounds described below.

Method D1.

Ethyl 5-acetoxy-*N*-[(2,3-dichloropyrid-5-yl)methyl]indole-2-carboxylate

90% yield. NMR: 1.27 (t, 3H), 2.26 (s, 3H), 4.28 (q, 2H), 5.85 (s, 2H), 7.12 (dd, 1H), 7.38 (s, 1H), 7.47 (d, 1H), 7.68 (d, 1H), 7.78 (d, 1H), 8.10 (d, 1H); m/z 409 $(M+H^+)$, 407.

Method D2.

Ethyl 5-acetoxy-*N*-(3-chloro-4-fluorobenzyl)indole-2-carboxylate

57% yield. NMR ($CDCl_3$): 1.37 (t, 3H), 2.33 (s, 3H), 4.35 (q, 2H), 5.74 (s, 2H), 6.90 (m, 1H), 7.00 (d, 1H), 7.05 (dd, 1H), 7.13 (dd, 1H), 7.26 (d, 1H), 7.36 (s, 1H), 7.22 (d, 1H).

Method E

Ethyl *N*-(3,4-dichlorobenzyl)-5-hydroxyindole-2-carboxylate

Sodium ethoxide (1.86 g) was added to a stirred solution of ethyl 5-acetoxy-*N*-(3,4-dichlorobenzyl)indole-2-carboxylate (5.55 g) in ethanol (50 ml) under an atmosphere of argon. The reaction was stirred at room temperature for 2 hours, then concentrated *in vacuo* and the residue acidified with aqueous hydrochloric acid (2 M) and

extracted with dichloromethane. Combined organic extracts were washed with water, saturated aqueous sodium chloride solution and dried. The solvent was removed *in vacuo* and the residue was triturated with hexane / diethyl ether to yield the product as a white solid (3.17 g, 92%). NMR: 1.26 (t, 3H), 4.25 (q, 2H), 5.75 (s, 2H), 6.81 - 6.91 (m, 2H), 6.98 (d, 1H), 7.19 (s, 1H), 7.29 (d, 1H), 7.38 (d, 1H) 7.50 (d, 1H), 9.06 (s, 1H); m/z 364 ($M+H^+$).

Example 4

Pharmaceutical Compositions

This Example illustrates, but is not intended to limit, representative pharmaceutical dosage forms of the invention as defined herein (the active ingredient being termed "Compound X"), for therapeutic or prophylactic use in humans:

Example A

(a)

Tablet I	mg/tablet
Compound X.	100
Lactose Ph.Eur	182.75
Croscarmellose sodium	12.0
Maize starch paste (5% w/v paste)	2.25
Magnesium stearate	3.0

(b)

Tablet II	mg/tablet
Compound X	50
Lactose Ph.Eur	223.75
Croscarmellose sodium	6.0
Maize starch	15.0
Polyvinylpyrrolidone (5% w/v paste)	2.25
Magnesium stearate	3.0

(c)

Tablet III	mg/tablet
Compound X	1.0
Lactose Ph.Eur	93.25
Croscarmellose sodium	4.0
Maize starch paste (5% w/v paste)	0.75
Magnesium stearate	1.0

(d)

Capsule	mg/capsule
Compound X	10
Lactose Ph.Eur	488.5
Magnesium	1.5

(e)

Injection I	(50 mg/ml)
Compound X	5.0% w/v
1M Sodium hydroxide solution	15.0% v/v
0.1M Hydrochloric acid	to adjust pH to 7.6
Polyethylene glycol 400	4.5% w/v
Water for injection	to 100%

(f)

Injection II	(10 mg/ml)
Compound X	1.0% w/v
Sodium phosphate BP	3.6% w/v
0.1M Sodium hydroxide solution	15.0% v/v
Water for injection	to 100%

(g)

Injection III	(1mg/ml, buffered to pH6)
Compound X	0.1% w/v
Sodium phosphate BP	2.26% w/v
Citric acid	0.38% w/v
Polyethylene glycol 400	3.5% w/v
Water for injection	to 100%

(h)

Aerosol I	mg/ml
Compound X	10.0
Sorbitan trioleate	13.5
Trichlorofluoromethane	910.0
Dichlorodifluoromethane	490.0

(i)

Aerosol II	mg/ml
Compound X	0.2
Sorbitan trioleate	0.27
Trichlorofluoromethane	70.0
Dichlorodifluoromethane	280.0
Dichlorotetrafluoroethane	1094.0

(j)

Aerosol III	mg/ml
Compound X	2.5
Sorbitan trioleate	3.38
Trichlorofluoromethane	67.5
Dichlorodifluoromethane	1086.0
Dichlorotetrafluoroethane	191.6

(k)

Aerosol IV	mg/ml
Compound X	2.5
Soya lecithin	2.7
Trichlorofluoromethane	67.5
Dichlorodifluoromethane	1086.0
Dichlorotetrafluoroethane	191.6

(l)

Ointment	ml
Compound X	40 mg
Ethanol	300 µl
Water	300 µl
1-Dodecylazacycloheptan-2-one	50 µl
Propylene glycol	to 1 ml

Note:

Compound X in the above formulations may comprise a compound as illustrated in Examples 1 to 3 herein.

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablets (a)-(c) may be enteric coated by conventional means, for example to provide a coating of cellulose acetate phthalate. The aerosol formulations (h)-(k) may be used in conjunction with standard, metered dose aerosol dispensers, and the suspending agents sorbitan trioleate and soya lecithin may be replaced by an alternative suspending agent such as sorbitan monooleate, sorbitan sesquioleate, polysorbate 80, polyglycerol oleate or oleic acid.